BLOOD CELL DYNAMICS IN A SIMPLE MODEL OF MICROVASCULAR NETWORKS

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ABSTRACT

The dynamic distribution of red blood cells (RBCs) plays a crucial role in cerebral oxygenation, metabolism and many brain disorders (e.g. Alzheimer, ischemic stroke). Despite the growing number of studies on blood rheology in microcirculation, there remains still a big need for detailed experimental data on RBCs distribution in network of microchannels. To our best knowledge, this is the first experimental study of blood cell dynamics in simple networks of microchannels (height less than 10μm) under well controlled conditions which allows a systematic understanding of effects caused by single blood cells.

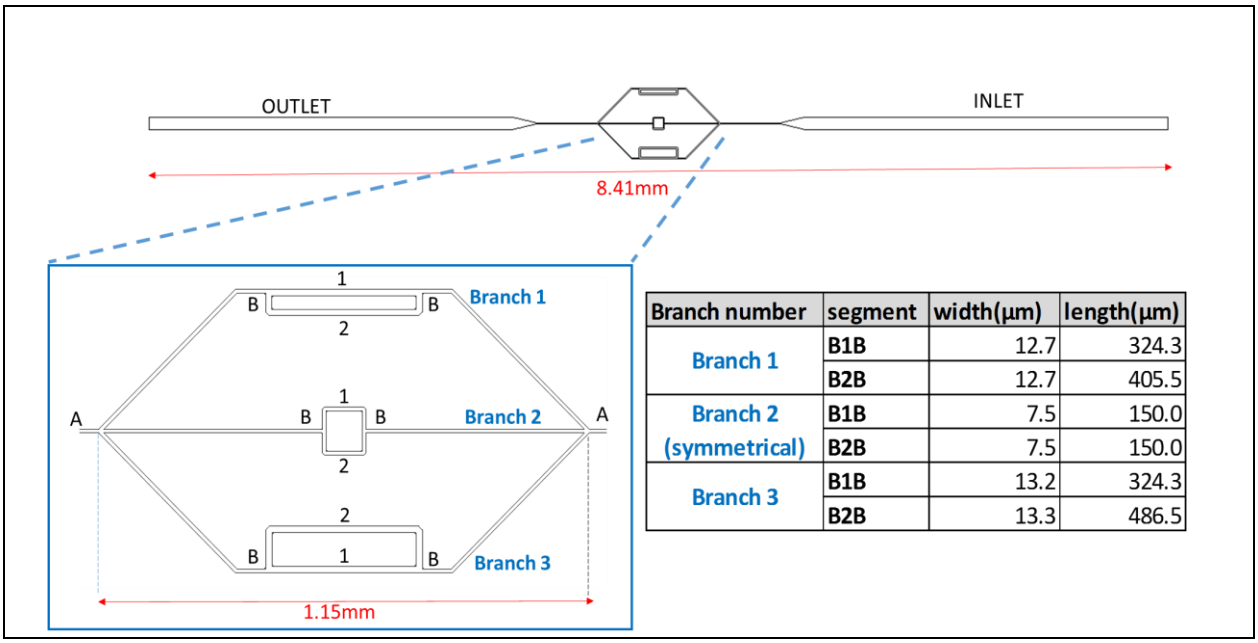
KEYWORDS: Blood cells, microvascular networks, microcirculation, cerebral blood flow

INTRODUCTION

Blood flow in microcirculation is a complex dynamic phenomenon which plays a crucial role in cerebral oxygenation, metabolism and many brain disorders. The effects of transient changes of local flow resistance due to the presence/absence of red blood cells (RBCs) are very complex even on a simple network [1,2]. Most available experimental data relies on qualitative observations. Some quantitative data were provided by Cybulski et al. [2] with droplets instead of RBCs and by Forouzan et al. [3] with RBCs flowing in a complex, irregular network of microchannels. There remains a big need for detailed quantitative experimental data to validate computational models [1]. In the present study we introduce a microdevice to enhance our insight into the complex flow phenomena in the microcirculation by following a bottom-up approach: starting from the analysis of RBC flow in simple networks, more complex features can be explained.

MATERIALS AND METHODS

The three branches of microchannels (height 7.5μm) (branches 1, 2 and 3, Figure 1) were designed to have identical hydraulic resistances for homogeneous Newtonian fluids, calculated using the formula suggested by [4]. The microdevice was fabricated using conventional soft lithography [3]. Blood (5ml sample) was collected from Specific Pathogen-free Swiss Landrace pigs using citrate as anticoagulant. Following the sample preparation described in [5], a solution of RBCs in Optiprep (Sigma-Aldrich, USA) and Gasp Buffer at hematocrit=2% was obtained. Cells were introduced at controlled rate by regulating the liquid level in the two reservoirs connected to the inlet/outlet of the microdevice. The microdevice was placed on the stage of a microscope (VWR, USA). Videos were recorded using a camera (Motic, China), with 1280x1024 pixels and a 10x magnification objective at 12 frames per second. A technique similar to [6] was used to measure the RBC velocity: line scans were sampled from the microchannels in branch 2 (Figure 2B), these line scans were stacked in a temporal sequence (Figure 2C) and the RBCs velocity was derived from the slope of the dark RBC traces in these stacks.



Branch number	segment	width(µm)	length(µm)
Branch 1	B1B	12.7	324.3
	B2B	12.7	405.5
Branch 2 (symmetrical)	B1B	7.5	150.0
	B2B	7.5	150.0
Branch 3	B1B	13.2	324.3
	B2B	13.3	486.5

Figure 1: Schematic of the microdevice: the three branches (1, 2, 3) were designed to have the same hydraulic resistances using the formula in [4]. Each branch has two microchannels, the study of the partition of RBCs at bifurcations is therefore possible in 3 different conditions since i) in branch 1 the lower microchannel is 25% longer then the upper microchannel, ii) branch 2 has two symmetrical microchannels, iii) the upper microchannel of branch 3 is 75% longer than the lower one. The microchannels had rectangular cross-sections, the height was 7.5µm in all the channels; lengths and widths are shown in the table. The microdevice was fabricated in polydimethylsiloxane (Sylgard 184, Dow Corning) using conventional soft lithography.

RESULTS AND DISCUSSION

We found that at low velocities (<50µm/s) the RBCs were almost homogeneously distributed in the three branches while at higher velocities (<1mm/s) RBCs concentrated mainly in branch 2 although the three branches were designed to have the same hydraulic resistances (data not shown). In the symmetric branch 2, the effects of a partial obstruction due to a white blood cell (WBC) stuck in the lower channels were observed (Figure 2). Within 10s we counted 12 RBCs in the upper microchannel and only 3 RBCs in the obstructed microchannel (i.e. number of dark lines in Figure 2C). When RBC1 (Figure 2A) reached the WBC, sudden changes of the RBC velocities were observed in both microchannels. Changes in the RBCs velocities and distributions, due to the obstruction in branch 2, were also observed in branch 1 and 3.

CONCLUSION

To our best knowledge, this is the first experimental study aiming at a systematic understanding of effects caused by single blood cells in simple networks of microchannels. In this study, the effects on RBC velocities and fluxes due to a stuck WBC could be quantified. Future developments will focus on the quantification of the effects introduced by steep changes of flow rate and hematocrit on RBCs distribution and velocities.

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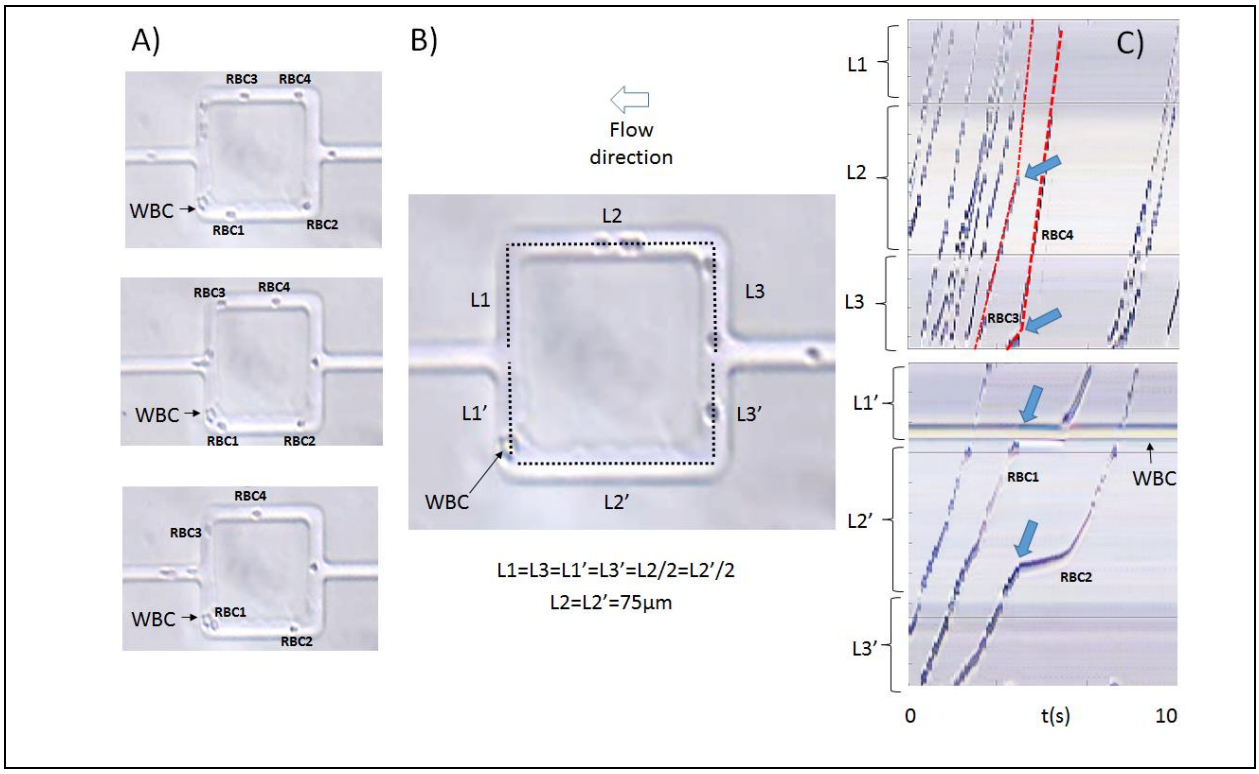

Figure 2: A) Sequence of frames (Δt=500ms) showing four RBCs, moving in branch 2 (i.e. RBC1, RBC2, RBC3, RBC4). The lower microchannel was partially obstructed by a white blood cell (WBC). Using the line scans in panel B (i.e. L1, L2, L3, L1', L2', L3') stacks of the moving RBCs were derived in C) for the non-obstructed microchannel (upper figure) and the obstructed one (lower figure) similarly to [6] (i.e. the slope of each dark line represents the RBC velocity associated to that line). When RBC1 reached the WBC (lower figure of panel A) it caused a sudden obstruction of the lower channel with immediate effects on the velocities of all the other RBCs. The blue arrows (panel C) indicate the change of slopes/velocities. For the upper microchannel the velocities increased i.e. from 44 to 104 µm/s for RBC3, from 15 to 84µm/s for RBC4. The velocity decreased in the lower microchannel i.e. for RBC2 from 19 to 4µm/s and then it increased again to 28µm/s after RBC1 moved again away from the WBC.

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